In-vitro antibacterial activity of *Allium humile*

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Abstract: This review aims to obtain the preliminary information regarding the inhibitory effects of the extracts of *Allium humile* on the test strains *B. subtilis, S. aureus, E. coli* and *P. aerugenosa.* The anti-bacterial activities of five solvent extracts viz. n-Hexane, Chloroform, Ethyl acetate, Methanol and Aqueous fractions of *Allium humile* were evaluated using disc diffusion technique. The extracts and fractions demonstrated significant anti-bacterial activity. Extract from Chloroform was the most potent against all the test organisms with the largest diameter of zone of inhibition. n-Hexane also showed considerable zone of inhibition. Ethyl acetate, Methanol and Aqueous fractions also exhibit slight inhibitory effects on both the gram-positive and gram negative test strains. [Academia Arena, 2010;2(6):83-86] (ISSN 1553-992X).

Keywords: *Allium humile*, Antibacterial, Zone of inhibition, chloroform extract, Inhibitory effect

Introduction :

Infectious diseases accounts for high proportion of health problems in the developing countries including India (Davies et al., 1994). Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created. The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease. This situation forced the researchers to search for new other antimicrobial substance from various sources including medicinal plants (Bauer et al., 1996). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Mann et al., 2008). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. Although chemical drugs are popular, however, herbal medicine continued to be practiced due to richness of certain plants in varieties of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids which have been reported to have antibacterial activities (Lewis and Ausubel, 2006, Cowan, 1999).

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 2006). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include roots, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are colleted in smaller quantities by the local communities and folk reveals for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 1996).

Considering the vast potentialities of plants as a source for anti-microbial drugs with reference to antibacterial agent, a systematic investigation was undertaken to screen the Allium humile for its antibacterial activity. Allium sp. has been used for centuries as remedies for human diseases because they contain components of therapeutic values. Allium humile is a perennial bulb having white flowers and belongs to Alliaceae family and grows naturally on slopes at high elevations in India. It is mainly found at the height of 1500-3000 meters of Alpine Himalayas of Uttarakhand, India, near moist rock, dry rock and steep slope with a strong preference of sunny site. Edible plant part used includes flowers, leaves, root and bulb. The leaf and bulb parts of this plant are used locally in the alleviation of inflammation and painful conditions (Farooquee et al, 2004). Leaves and inflorescences are also used as seasoning agents. Although no specific mention of medicinal uses has been for this species, member of this genus are in general very healthy additions to the diet. In this study, we investigated the antibacterial activity of petroleum ether, ethyl acetate, chloroform, methanol and aqueous extracts of A. humile against a panel of Gram positive and Gram negative bacteria. As per our knowledge, the antibacterial activities of this plant have been reported for the first time.

Materials and Methods:

Plant material:

Fresh disease free leaves and seeds of the plant were collected from Deovan, Chakrota, Uttarakhand, India, from an altitude of 1575 meter. Leaves were 4-7cm long, 4-5 mm wide. The leaves were washed

thoroughly several times with running water and once with sterile distilled water. The leaf material was then air-dried on a sterile blotter under shade. A voucher specimen and seed of the plant has been deposited in the herbarium of National Bureau of Plant Genetic Resources (NBPGR), Pusa, Delhi, India. The National Identity number of Allium humile is IC 567643.

Preparation of Extracts:

Solvent Extracts:

The thoroughly washed, shade dried sample (45 days) was subjected to the soxhlet extractor. The solvents employed for the fulfillment of this research were n-Hexane (68^0C), Chloroform (61.2˚C), Ethyl acetate (76˚C), Methanol (64.6˚C) and aqueous (100˚C). The extracts were separated by running a soxhlet assembly where solvents were applied as per their polarity at their boiling point. All the extracts were concentrated using Rotary flash Evaporator and preserved at 4˚C in air-tight bottles until further use. All the extracts were then subjected to anti-bacterial activity assay.

Microbial Cultures:

Four different bacterial strains were employed for the successful accomplishment of the study viz. Bacillus subtilis (MTCC 441), Staphylococcus aureus (MTCC 96), E. coli (MTCC 739) and Pseudomonas aeruginosa (MTCC 429). These strains were tested for their degree of resistance towards the different extracts of the plant sample. The strains were collected from Institute of Microbial Technology, Chandigarh, India. All the test strains were maintained on Nutrient Agar slopes (Hi-Media) and were subjected to anti-bacterial activity assay.

Anti-bacterial Assay:

Antimicrobial activity was carried out using disc-diffusion method (Bauer et al., 1996). The extracts were dissolved in DMSO (1% v/v) to yield the final concentration of 100 mg/mL. Sterile discs (Himedia, India) were impregnated with the prepared extracts. For the preparation of the inoculation, the tested bacteria were cultured in nutrient broth at 37°C for 24 h and 0.5 of the McFarland unit, which was used (Barry and Thornsberry, 1985). One hundred microliters of prepared culture were spread on the surface of nutrient agar (Hi-Media) for bacterial pathogens. The plates were kept at ambient temperature for 30 min to enable diffusion of extracts and then incubated at 37°C for 24 h. Discs impregnated with only solvents were used as negative controls and antibiotic discs of streptomycin (10 µg/disc) (Hi-Media, India) for bacteria was used as positive controls. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. Each experiment was repeated at least three times and mean of the diameter of inhibition zones was calculated.

Phytochemical Screening

The n-Hexane, Chloroform, Ethyl acetate, Methanol and Aqueous extracts of A. humile were subjected to qualitative chemical tests for the indentification of various plant constituents like tannins, polyphenols, flavonoids, alkaloids, steroids and saponins. Two milliliters of each extract was measured into a test tube for each of the tests and concentrated by evaporating extractant in a water bath.

Results

Extract Yield

Percentage extraction for the different solvents used was 54% (water), 46% (hexane), 40% (ethyl acetate), 34% (methanol) and 24% (chloroform). Water is a universal solvent and is generally used in traditional settings to prepare the plant decoctions for health remedies. All the extracts were acidic in nature (pH values ranging between 5.0-5.5). The acidity combined with bioactive components might enhance the antimicrobial activity of the extracts against the bacteria.

Anti-bacterial Assay

The different extracts and fractions of *Allium humile* demonstrated significant anti-bacterial activity. Chloroform was the most potent against most of the test organisms with largest diameter of zone of inhibition i.e. 18mm against B. subtilis. n-Hexane also showed considerable zone of inhibition viz. 13mm against E.coli and P.aeruginosa. The plant extracts and their antimicrobial activity on the given bacterial strains are shown in (Figure 1).

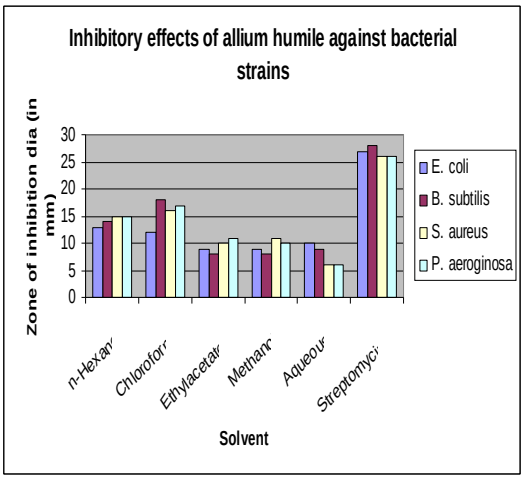

Figure 1: Graphical representation of inhibitory effect of different extract of *Allium humile* against test bacterial strains

In vitro antimicrobial study indicated maximum range (61-65%) for chloroform extract of the four test strains, while the minimum range of inhibitory activity (23-28%) was exhibited by different extract in different solvent systems (aqueous for Staphylococcus aureus and Pseudomonas aeruginosa, ethyl acetate for Bacillus subtilis, methanol for Bacillus subtilis). However, no extract (of all the solvents used) showed any antibacterial activity against M. luteus (Table 1).

Phytochemical Screening

Qualitative phytochemical investigation revealed that the extracts contained some phytoconstituents. Saponins, tannins, alkaloids and flavonoids are present in the acetone extracts; tannins, alkaloids and flavonoids are found in the methanol extracts; alkaloids and flavonoids in water; and hexane extracts and saponins and tannins in dichloromethane extracts (Table 2). These bioactive components including thiocynate, nitrate, chloride and sulphates, beside other water soluble components which are naturally occurring in most plant materials, are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the anti-microbial property to plants.

Table 1: Zone of inhibition diameter (in mm) of different extracts of *Allium humile*

	Extracts of *Allium humile*					
Test pathogens	**Methanol**	**n-Hexane**	**Chloroform**	**Aqueous**	**Ethyl Acetate**	**Streptomycin**
E. coli	9	13	12	10	9	27
B. subtilis	8	14	18	9	8	28
S. aureus	11	15	16	6	10	26
P. aeruginosa	10	15	17	6	11	26
Micrococcus luteus.	-	-	-	-	-	23

Table 2. Phytochemical analysis of different extract of *Allium humile*

		Extracts of *Allium humile*				
Tests		**Methanol**	**n-Hexane**	**Chloroform**	**Aqueous**	**Ethyl Acetate**
1.	**Steroids**					
i. a	Salkowski test	(+)	(+)	(-)	(-)	(-)
ii.	Gilberman-Buchard's test	(+)	(+)	(-)	(-)	(-)
2.	**Alkaloids**					
i.	Wagner's test	(-)	(-)	(+)	(+)	(+)
ii.	Hager's test	(-)	(-)	(+)	(+)	(+)
3.	**Phenolic and Flavonoid compounds**					
i.	Vanilin-HCL test	(-)	(-)	(+)	(-)	(+)
ii.	Ferric chloride test	(+)	(+)	(+)	(-)	(-)
iii.	Zinc hydrochloric acid reduction test	(-)	(-)	(-)	(-)	(-)
4.	**Tannins**	(-)	(-)	(+)	(+)	(+)
5.	**Saponins**	(-)	(-)	(+)	(+)	(+)

Discussion

The higher resistance of Gram-negative bacteria to plant extracts has previously been documented and related to thick murein layer in their outer membrane, which prevents the entry of inhibitor substances into the cell (Martin, 1995; Brantner et al., 1996; Palombo and Semple, 2001; Tortora et al., 2001; Matu and van Staden, 2003). Similarly, our results indicated that the antibacterial activities of the extracts were more pronounced on Gram positive than on Gram-negative bacteria. The chloroform extract of leaf of Allium humile has shown the maximum antibacterial activity regardless of the solvent system. It also showed maximum inhibitory activity against all the test bacterial strains except E.coli against which the n-hexane extract had shown highest activity. The antimicrobial activity exhibited by various extracts of leaf was, however, less than the standard drugs used.

Acknowledgement

We wish to show gratitude to the Management, Sardar Bhagwan Singh (P.G) Institute of Biomedical Sciences and Research, Balawala, Dehradun, India for providing necessary research facilities. We duly acknowledge the Director, Uttarakhand Council of Science and Technology (UCOST) for financial assistance.

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15/04/2010